

## Materials Science and Technology Analytical Methods

# Microanalysis and the FBI's Investigation of the 2001 Anthrax Attacks

*"Amerithrax"  
investigation asks  
Sandia to perform  
detailed forensic analyses  
to help determine  
methods of manufacture*

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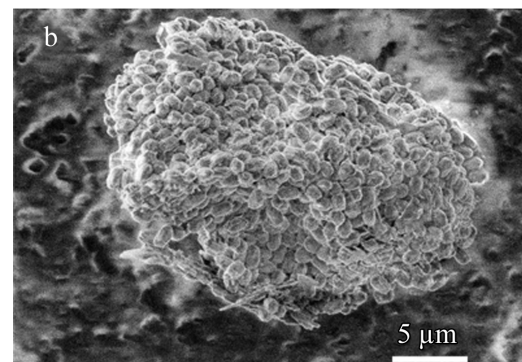
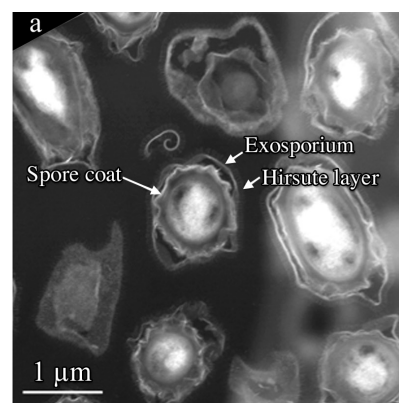
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The anthrax attacks of 2001 in the U.S. killed five, sickened twenty-two others, and caused a significant disruption of mail and other government facilities. Although some of the attack materials (*Bacillus anthracis*) were recovered in powder form in sealed envelopes, the US Federal Bureau of Investigation (FBI) was unprepared to perform the needed forensic analyses on these bio-weapon substances. In particular, they realized that analysis from the micro- to nano-scale was a key missing piece of their capabilities. As a result, Sandia was asked to analyze the materials from the attacks and report findings by early 2002; Sandia was able to submit an initial report within a few months. A more detailed investigation was carried out subsequently, and finally concluded in 2008. Over 200 samples of *B. anthracis* were analyzed in an attempt to discern the method of manufacture of the attack materials. After so many years, Sandia is now able to discuss this previously classified research.

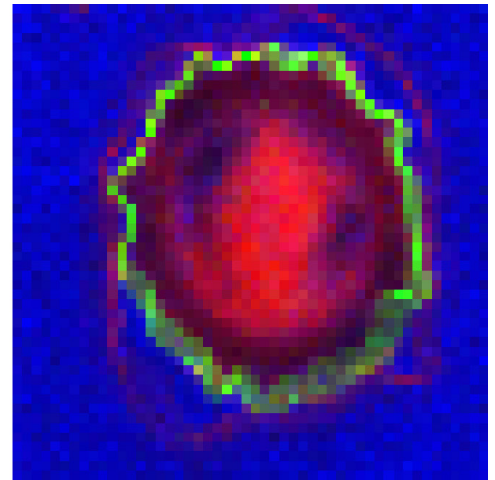
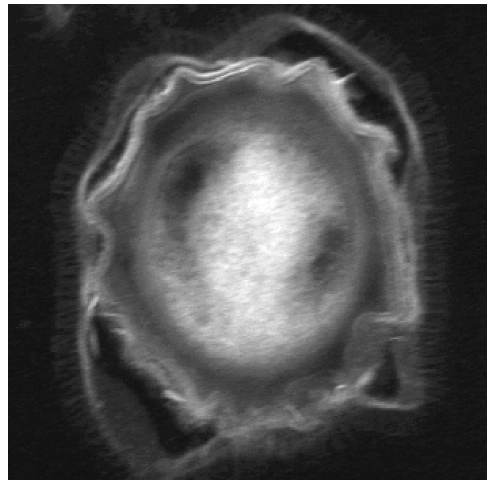
Sandia's long expertise in the development and application of microanalysis techniques, available only at Sandia at the time, led the FBI to ask for its involvement in their investigation. They asked Sandia to answer several critical questions, including: Was the *B. anthracis* treated to make it more lethal? Were the materials in the various attacks from the same source? Three primary microanalysis techniques, combined with multivariate statistical analysis [References 1-3], were used to answer these questions: (1) x-ray spectral imaging, where a complete x-ray spectrum is acquired from each point in an array, (2) scanning electron microscopy (SEM), and (3) scanning transmission electron microscopy (STEM). Figure 1a is a STEM image of a fixed, stained, and



**Figure 1:** a) Annular dark-field STEM image of a fixed and stained, microtomed section of *B. Anthracis* from the *New York Post* letter. b) SEM image of a clump of *B. Anthracis* spores from the Leahy letter.

microtomed section of *B. anthracis* spores from the letter sent to the *New York Post*. The spores had not been treated with silicon oxide (Si-O) nanoparticles that have been typically used as dispersal agents to make the substance more lethal. Figure 1b is an SEM image of a clump of spores from the letter sent to Senator Leahy where there is no extraneous material visible at these length scales. Further analyses of material from the letter sent to Senator Daschle show similar results. Detailed microanalysis, as shown in Figure 2, did indicate the presence

**Figure 2:** Annular dark-field STEM image of a single spore from the *New York Post* letter (left), and microanalysis results (right) showing that Si-O (green) is present on an internal spore structure, namely the spore coat. Red corresponds to the fixing/staining materials (osmium, uranium, and lead) while blue corresponds to the plastic in which the spores were embedded for microtoming. Image widths are 1.5  $\mu\text{m}$ .



of Si-O in the attack materials. However, it was associated with an internal structure of the spores, and was thus related to the growth method, rather than added post-sporulation for enhanced dispersion. The chemical signature of the spore coat of the attack materials included not only silicon and oxygen as major elements, but also minor amounts of iron and tin. All of the attack materials examined by Sandia shared this common signature, thus indicating that they were most likely produced by the same method and probably from the same source.

## References

1. P.G. Kotula, M.R. Keenan and J. R. Michael, "Automated Analysis of SEM X-Ray Spectral Images: A Powerful New Microanalysis Tool," *Microsc. Microanal.* **9**, 1-17 (2003).
2. P.G. Kotula and M.R. Keenan, "Application of Multivariate Statistical Analysis to STEM X-Ray Spectral Images: Interfacial Analysis in Microelectronics," *Microsc. Microanal.* **12**, 538-544 (2006).
3. L.N. Brewer, J. A. Ohlhausen, P.G. Kotula, and J.R. Michael, "Forensic analysis of bioagents by X-ray and TOF-SIMS hyperspectral imaging," *Forensic Science International* **179** 98-106 (2008).